SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS -
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT This document has been approved for public release and sale; its distribution is unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION U.S. Army Medical Research Institute of Infectious Diseases 6b. OFFICE SYMBOL (If applicable) SGRD-UIP-B	7a. NAME OF MONITORING ORGANIZATION U.S. Army Medical Research and Development Command
6c. ADDRESS (City, State, and ZIP Code) Ft. Detrick Frederick, MD 21701-5011	7b. ADDRESS (City, State, and ZIP Code) Ft. Detrick Frederick, MD 21701-5011
8a. NAME OF FUNDING/SPONSORING ORGANIZATION 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification) Evaluation of Neutralizing Antibodies to Types A, B, E, and F Botulinum Toxins in Sera from Human Recipients of Botulinum Pentavalent (ABCDE) Toxoid

12. PERSONAL AUTHOR(S)
Lynn S. Siegel

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Interim	FROM _____ TO _____	28 February 89	10

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Twenty-five sera from personnel immunized with botulinum pentavalent toxoid (ABCDE) had titers of neutralizing antibodies to type A [5.7-51.6 international units (IU/ml], type B (0.75-18 IU/ml), and to type # (0.61-10 IU/ml) botulinum toxins. Titers for one type could not be used to predict titers for another type in individuals receiving the toxoid. Cross-neutralizing antibodies to type F botulinum toxin were not detected (<0.0125 IU/ml).

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION
22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code) / 22c. OFFICE SYMBOL

Evaluation of Neutralizing Antibodies to Types A, B, E, and F Botulinum Toxins in Sera from Human Recipients of Botulinum Pentavalent (ABCDE) Toxoid

LYNN S. SIEGEL

Department of Toxinology
Pathology Division
U. S. Army Medical Research Institute of Infectious Diseases
Fort Detrick
Frederick, Maryland 21701-5011

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the author do not purport to reflect the positions of the Department of the Army or the Department of Defense.

All correspondence to: Dr. Lynn Siegel
Pathology Division
USAMRIID, Fort Detrick
Frederick, MD 21701-5011
Telephone: (301) 663-7211

ABSTRACT

Twenty-five sera from personnel immunized with botulinum pentavalent toxoid (ABCDE) had titers of neutralizing antibodies to type A [5.7-51.6 international units (IU)/ml], type B (0.75-18 IU/ml), and to type E (0.61-10 IU/ml) botulinum toxins. Titers for one type could not be used to predict titers for another type in individuals receiving the toxoid. Cross-neutralizing antibodies to type F botulinum toxin were not detected (<0.0125 IU/ml).

There are seven types of Clostridium botulinum, designated A-G, each type producing a pharmacologically similar but immunologically distinct neurotoxin. Immunization with botulinum toxoid has been used for over 40 years to protect laboratory personnel at risk for botulism due to contact with the neurotoxins. The botulinum toxoid currently distributed by the Centers for Disease Control is pentavalent, containing Formalin-inactivated botulinum toxins of types A, B, C, D, and E, adsorbed to aluminum phosphate (4). Toxoids against types F and G are not currently available for human use. However, it has been reported that large quantities of antibody to type E neurotoxin can neutralize small quantities of type F neurotoxin (2, 3, 12, 15). This is of particular interest because type F botulinum neurotoxin does cause human disease, both food-borne (11, 12) and infant (10) botulism. Furthermore, there are single strains of C. botulinum that produce type F along with another type of neurotoxin; A + F (7), including a strain isolated from a patient with food-borne botulism (5), and B + F isolated from a case of infant botulism (9). We therefore investigated the possibility that sera from individuals receiving the pentavalent toxoid and having high titers of neutralizing antibodies to type E neurotoxin could also neutralize type F neurotoxin.

As previously described (13), sera obtained from individuals immunized with botulinum pentavalent (ABCDE) toxoid were tested for neutralizing antibodies to type A or B botulinum toxin, using a mouse bioassay. Serum samples that had a high A and B titer (arbitrarily defined as > 5 and > 0.7 international units/milliliter, respectively) were assayed for neutralizing antibodies to type E and to type F botulinum toxins. (One international unit [IU] is defined as the amount of antibody neutralizing 10,000 mouse intraperitoneal 50% lethal doses [LD_{50}] of type A, B, C, D, or F botulinum toxin or 1,000 mouse intraperitoneal LD_{50} of type E [14].) For comparison, note that the Centers for Disease Control

recommends against administration of a booster immunization to any individual having a titer of approximately 0.25 IU/ml (1:16) or greater for the types of botulinum toxin to which he or she is at risk (4). Typically, the antibody response to the B component of the toxoid is the poorest (1, 6).

The concentration of type E or F toxin used in the assay was that which was neutralized by 0.0125 IU/ml of the homologous type of World Health Organization International Standard antitoxin. For type E, fourfold dilutions of serum samples (1/16 to 1/1,024) were mixed with an equal volume of standardized toxin. In the type F neutralization test, sera were initially tested without dilution and at 1/4 to 1/64, but in subsequent assays only undiluted serum was used. For both E and F, the toxin-serum mixtures were incubated for 1 h at room temperature, and then 0.2 ml was injected intraperitoneally into each of eight mice. The animals were observed for four days for deaths. The concentration of neutralizing antibodies in the serum was calculated relative to the homologous World Health Organization International Standard antitoxin (equine for E, rabbit for F) which was included in each test, and results are reported as IU/ml. Undiluted sera that did not protect mice from death are reported as <0.0125 IU/ml for type F.

Results are shown in Table 1. In this group of 25 sera, the A titers ranged from 5.7 to 51.6 IU/ml and the B titers from 0.75 to 18 IU/ml, but the E titers were lower than anticipated, 0.61 to 10 IU/ml (Table 1). Ranking the sera in ascending order by type A titer did not demonstrate a corresponding increase in titer for type B or type E. For the 25 serum samples assayed, there was no correlation between the neutralizing antibody titers for types A, B, and E botulinum toxins, and the titer to one toxin type could not be used to predict that to another type.

Previous investigations with animal sera demonstrated that large

quantities of type E antitoxin would neutralize small quantities of type F toxin. Moller and Scheibel (12) reported that equine antitoxin from the Microbiological Research Establishment, Porton, England, neutralized 3,200 mouse minimal lethal doses (MLD) of type E toxin and 2 MLD of type F toxin (Langeland strain). Antitoxins from the Pasteur Institute, Paris, produced analogous results (12). A quantity of E antitoxin (Connaught, equine) that neutralized 4,000 MLD of type E toxin also neutralized 10 MLD of type F Langeland (2). Two to three MLD of type F toxin from strain 202F, a nonproteolytic strain isolated from marine sediments on the Pacific Coast of the United States, was neutralized by a volume of type E antitoxin from the Centers for Disease Control which neutralized 1,000 MLD of type E toxin (3). Rabbit antitoxin prepared against purified type E neurotoxin which neutralized 2,000 LD_{50} of type E toxin also neutralized 5 LD_{50} of type F toxin (15).

In the study reported here for human immune sera, titers for type E ranged from 0.61 to 10 IU/ml. However, neutralizing antibodies to type F were not detected (<0.0125 IU/ml) in each of the sera. Thus, human sera at 10 IU/ml for type E (1 ml of serum can neutralize 10,000 LD_{50} of type E botulinum toxin) failed to neutralize 125 LD_{50} of type F botulinum toxin. Titrations to determine the exact number of LD_{50} of type F toxin that the sera could neutralize, if any, were precluded by the lack of sufficient volumes of sera. However, our purpose was to determine whether immunization of personnel with the pentavalent toxoid had elicited levels of cross-neutralizing antibody to type F neurotoxin that could be considered protective. The correlation between the level of neutralizing antibody in the serum and the ability to withstand an exposure to botulinum toxin is, of course, not known for humans. Values used as "satisfactory" titers in humans for

types A, B, C, D, and E have been extrapolated from animal studies, and were established to indicate that an individual had responded to the pentavalent immunogen (6). These "satisfactory" levels are twice the lowest titer that can be measured using the mouse bioassay (1). Using purified type F monovalent toxoid to immunize guinea pigs, Hatheway demonstrated a relationship between antibody levels and the ability of the animals to survive challenge with 10^5 LD_{50} of type F toxin (8). All guinea pigs having antibody concentrations greater than 0.04 U/ml survived challenge, as did 50 to 100% of the animals with titers of 0.01 to 0.04 U/ml. However, groups of animals with antibody levels that were undetectable (<0.01 U/ml) had a survival rate of less than 50%, after challenge. Thus, antibody levels in guinea pigs that are protective for type F toxin are comparable to those previously established for types A, B, C, D, and E. Similar extrapolation of these data for type F to humans would indicate that 0.025 IU/ml is a "satisfactory" titer.

The neutralization of small amounts of type F botulinum toxin by type E antitoxin (2, 3, 12, 15) is important biochemically because it indicates that the two neurotoxic proteins share some common epitopes, and therefore have a degree of structural similarity. However, this cross-neutralization is of little practical significance.

I thank James Osmolski and Karen Saldana for outstanding technical assistance.

LITERATURE CITED

1. **Cardella, M. A.** 1964. Botulinum toxoids, p. 113-130. *In* K. H. Lewis and K. Cassel, Jr. (ed.), Botulism: proceedings of a symposium. U. S. Department of Health, Education, and Welfare, Public Health Service, Cincinnati.

2. **Dolman, C. E., and L. Murakami.** 1961. Clostridium botulinum type F with recent observations on other types. J. Infect. Dis. **109**:107-128.

3. **Eklund, M. W., F. T. Poysky, and D. I. Wieler.** 1967. Characteristics of Clostridium botulinum type F isolated from the Pacific coast of the United States. Appl. Microbiol. **15**:1316-1323.

4. **Ellis, R. J.** 1982. Immunobiologic agents and drugs available from the Centers for Disease Control: descriptions, recommendations, adverse reactions, and serologic response, 3rd ed. Centers for Disease Control, Atlanta.

5. **Fernandez, R. A., Ciccarelli, A. S., Arenas, G. N., and D. F. Gimenez.** 1986. First Clostridium botulinum subtype Af outbreak. Revista Argentina de Microbiologia **18**:29-32.

6. **Fiock, M. A., M. A. Cardella, and N. F. Gearinger.** 1963. Studies on immunity to toxins of Clostridium botulinum. IX. Immunologic response of man to purified pentavalent ABCDE botulinum toxoid. J. Immunol. **90**:697-702.

7. **Gimenez, D. F., and A. S. Ciccarelli.** 1970. Studies on strain 84 of Clostridium botulinum. Zentrabl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig. Reihe A **215**:212-220.

8. **Hatheway, C. L.** 1976. Toxoid of Clostridium botulinum type F: Purification and immunogenicity studies. Appl. Environ Microbiol. **31**:234-242.

9. **Hatheway, C. L., and L. M. McCroskey.** 1987. Examination of feces and serum for diagnosis of infant botulism in 336 patients. J. Clin. Microbiol. **25**:2334-2338.

10. **Hoffman, R. E., Pincomb, B. J., Skeels, M. R., and M. J. Burkhart.** 1982. Type F infant botulism. Am. J. Dis. Child. **136**:270-271.

11. **Midura, T. F., G. S. Nygaard, R. M. Wood, and H. L. Bodily.** 1972. Clostridium botulinum type F: Isolation from venison jerky. Appl. Microbiol. **24**:165-167.

12. **Moller, V., and I. Scheibel.** 1960. Preliminary report on the isolation of an apparently new type of Cl. botulinum. Acta Pathol. Microbiol. Scand. **48**:80.

13. **Siegel, L. S.** 1988. Human immune response to botulinum pentavalent (ABCDE) toxoid determined by a neutralization test and by an enzyme-linked immunosorbent assay. J. Clin. Microbiol. **26**:2351-2356.

14. **Smith, L. DS.** 1977. Botulism: The organism, its toxins, the disease, p.78. Charles C. Thomas, Springfield, Ill.

15. **Yang, K. H., and H. Sugiyama.** 1975. Purification and properties of Clostridium botulinum type F toxin. Appl. Microbiol. **29**:598-603.

TABLE 1. Neutralization titers to types A, B, E, and F botulinum toxins for 25 individuals

Neutralization Titer (IU^a/ml)			
Type A	Type B	Type E	Type F
5.74	1.28	2.53	<0.0125
5.74	4.07	9.16	<0.0125
6.01	2.32	9.16	<0.0125
6.45	2.18	2.26	<0.0125
7.24	5.12	2.26	<0.0125
9.12	3.23	9.16	<0.0125
10.2	2.03	0.69	<0.0125
11.5	2.18	5.65	<0.0125
12.0	3.46	6.73	<0.0125
12.9	2.03	0.62	<0.0125
12.9	2.87	2.76	<0.0125
14.5	0.75	1.10	<0.0125
14.5	6.64	2.85	<0.0125
16.3	3.23	2.26	<0.0125
18.2	2.87	3.20	<0.0125
18.2	2.87	6.40	<0.0125
22.2	4.07	1.74	<0.0125
23.0	3.23	1.74	<0.0125
25.6	2.75	6.40	<0.0125
35.2	3.23	6.40	<0.0125
41.0	3.23	0.61	<0.0125
41.0	4.18	7.07	<0.0125
41.0	5.75	10.0	<0.0125
41.0	5.75	10.0	<0.0125
51.6	18.2	7.07	<0.0125

[a] One International Unit (IU) is the amount of antibody neutralizing 10,000 mouse intraperitoneal 50% lethal doses of type A, B, or F botulinum toxin, or 1,000 mouse intraperitoneal 50% lethal doses of type E.